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# Combating infectious diseases in aquaculture with an original probiotic product

Meagan Hamblin
*University of Rhode Island*, meagan_hamblin@my.uri.edu

Saebom Sohn
*University of Rhode Island*, bomisohn@gmail.com

Christine Ahn Dao
*University of Rhode Island*

Marta Gomez-Chiarri
*University of Rhode Island*

David R. Nelson
*University of Rhode Island*

*See next page for additional authors*

# Combating infectious diseases in aquaculture with an original probiotic product


**Meagan Hamblin[1], Christine Dao[1], Saebom Sohn[2], Marta Gomez-Chiarri[2], David Nelson[3], David Worthen[1], David Rowley[1]**

THE UNIVERSITY OF RHODE ISLAND

[1] Department of Biomedical and Pharmaceutical Sciences, University of Rhode Island, Kingston, RI 02881
[2] Department of Fisheries, Animal and Veterinary Sciences, University of Rhode Island, Kingston, RI 02881
[3] Department of Cell and Molecular Biology, University of Rhode Island, Kingston, RI 02881


## INTRODUCTION

Bacterial infections create substantial economic losses for commercial shellfish hatcheries. Hence, an effective product is needed to protect larvae, which are most susceptible to infection. Previous studies have shown that a native Rhode Island bacterium, *Bacillus pumilus* RI06-95, protects oyster larvae against pathogenic *Vibrio* species. In this study, a freeze-dried formulation of this probiotic microbe was developed. Stability, dispersion in seawater and pilot-scale hatchery studies were performed to further investigate this product for use in aquaculture facilities.

## MATERIALS and METHODS

**FIGURE I. Formulation Procedure**

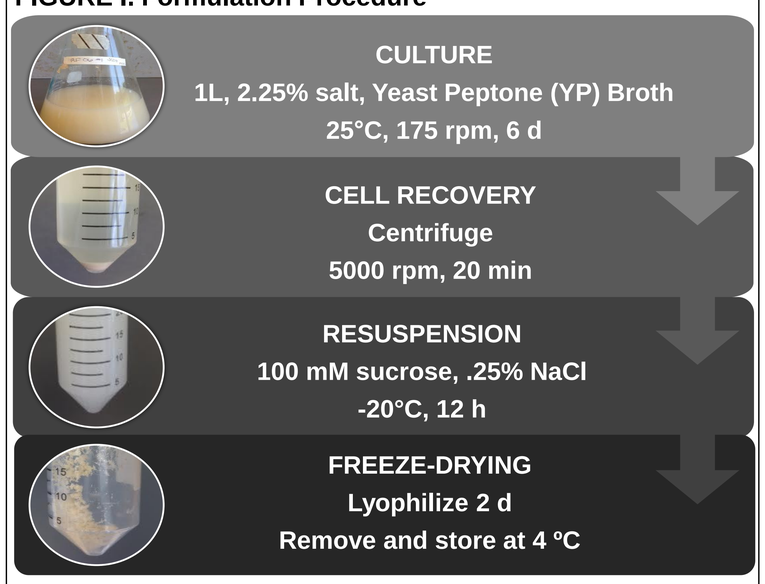
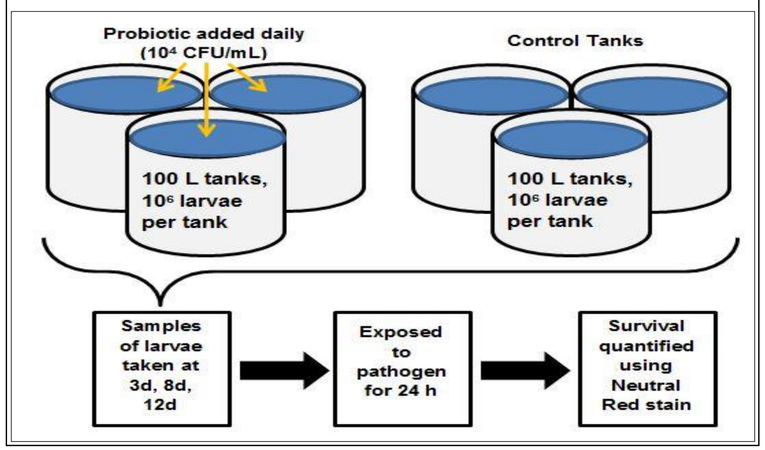
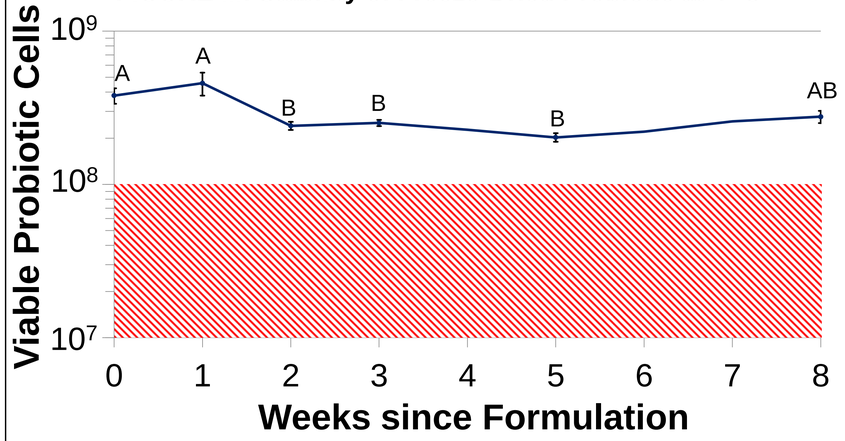



**FIGURE II. Hatchery Trial Procedure**




## RESULTS

**FIGURE III: Stability of Freeze-Dried Probiotic at 4 °C**




- Product viability (CFU/ml) determined by spot-plating method
- Minimum effective concentration is $10^8$ CFU/mL in each single-use tube

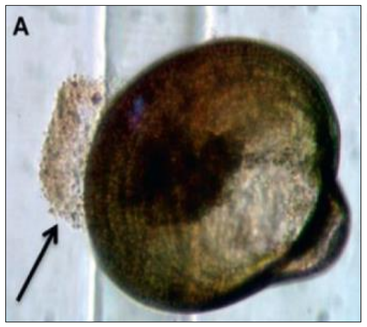



**Image A:** Infected oyster larva

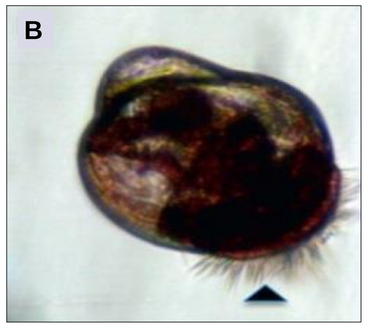
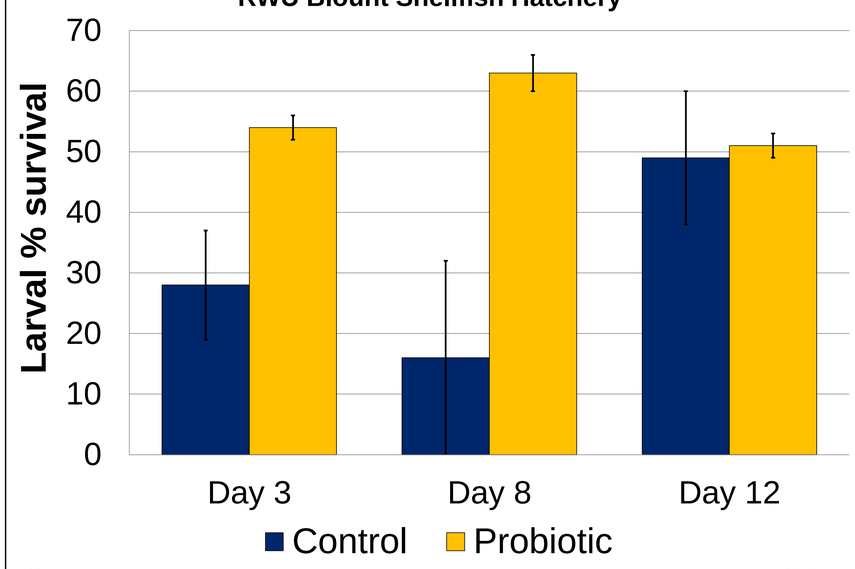



**Image B:** Healthy oyster larva

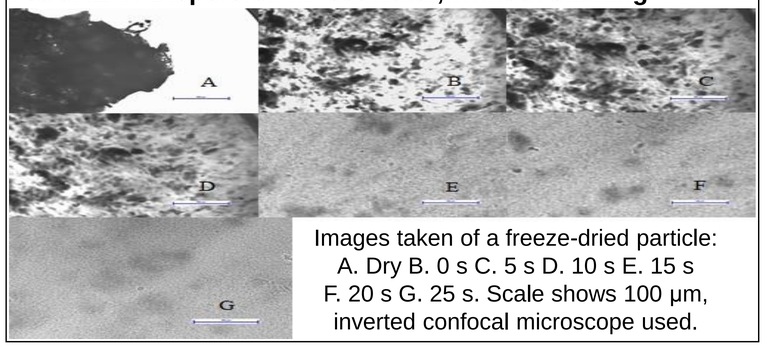
**FIGURE IV: Pilot-Scale study of the formulated probiotic at RWU Blount Shellfish Hatchery**




**Corresponding control groups not exposed to pathogen all showed 85% or higher survival.**

## DISPERSION

**FIGURE V: Dispersion in seawater, without shaking**




Images taken of a freeze-dried particle:
A. Dry B. 0 s C. 5 s D. 10 s E. 15 s
F. 20 s G. 25 s. Scale shows 100 µm,
inverted confocal microscope used.

## CONCLUSIONS and DISCUSSION

**Based on the hatchery trial results:**

- Adding this product to tanks is safe for larvae
- The probiotic product decreases larval mortality caused by *Vibrio* infections
- The product provides significant protection of larvae and may be used in a commercial hatchery

**Based on the stability and dispersion study:**

- The product remains stable for two months or longer under standard refrigeration conditions
- The product quickly disperses in sea water without added energy

**Future experiments include:**

- Quantitative study of the dispersion
- Pilot-scale hatchery studies at other locations

### References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# Poster Summary

The shellfish aquaculture industry is of great environmental and financial importance, particularly in the state of Rhode Island as well as throughout the world. While this industry continues to grow to meet worldwide demand, it is constrained by infectious diseases, such as Vibriosis, which is caused by pathogenic *Vibrio* species bacteria. Shellfish hatcheries, facilities that rear larvae, are most impacted by infectious disease and are seeking an effective means to prevent larval tank infections. Probiotics are beneficial bacteria which may compete with, and prevent pathogenic bacteria from colonizing a host and causing infection. By using a probiotic strain of bacteria, *Bacillus pumilus* RI06-95 that was previously identified by Krim et.al., a formulation of a marine probiotic product was created and evaluated. By lyophilizing, or freeze-drying, the bacteria in 10 mL of 100 mM sucrose, 2.25% NaCl, DI water, then storing at 4 degrees Celsius, a standard household refrigerator temperature, an original formulation of this marine probiotic was created.

The product's stability was studied over a span of eight weeks; triplicate samples reconstituted, diluted and spot-plated at each time point reveal that the product maintains the necessary minimum concentration of viable cells, $10^8$ CFU/mL, for at least eight weeks when kept refrigerated. In addition, the product's dispersion in sea water was qualitatively observed using a confocal light microscope, by capturing images of the dispersion in five seconds intervals. The product appears to readily disperse within twenty-five seconds of being added to sterile salt water, without any shaking or additional energy. The efficacy of the product was also observed in a pilot-scale hatchery study, in which three larval tanks received the probiotic product daily, and three control tanks received no treatment. Samples of larvae from each tank were taken at day three, eight and twelve or treatment and exposed to the pathogen *Vibrio tubiashii* RE22. After twenty-four hours the larval mortality was quantified using neutral red stain. The pathogen challenge revealed that a significant protective effect was seen at days three and eight of receiving the probiotic treatment. Ultimately, this study successfully investigated the stability, dispersion and efficacy of the product. Moving forward, we hope to perform a quantitative study of the dispersion, and continue to investigate the efficacy of this product in multiple pilot-scale studies at different hatchery locations.